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Anti-P30 IgA antibodies as prenatal markers of congenital toxoplasma infection

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SUMMARY

This study extends a previous study and confirms that the detection of anti-P30 IgA antibodies is very helpful in the diagnosis of acute acquired or congenital toxoplasmosis. Moreover, we demonstrate that an anti-P30 IgA response can be mounted in the fetuses infected by *Toxoplasma gondii* during their intra-uterine life as early as week 23 of gestation. A double-sandwich ELISA described in our previous work was used to detect anti-P30 IgA antibodies in 1378 human serum samples collected from 551 patients, including 162 fetuses whose mothers had been infected by *T. gondii* during pregnancy, 46 congenitally infected and 90 uninfected newborns and 253 women suspected of having been infected during pregnancy, including the mothers of fetuses and newborns previously described. Anti-P30 IgA antibodies were detected in all cases of acute toxoplasmosis but in no case of chronic toxoplasmosis: in the majority of cases, the IgA antibody titre fell below cut-off in 3–9 months. Among the 46 congenitally infected newborns, anti-P30 IgA antibodies were detected in sera of 41 infected newborns (38 at birth, two in the first months of life, one in the seventh month of life), while anti-P30 IgM antibodies were detected in only 30 cases at birth and in one case during the first month of life. Among 162 fetuses, anti-P30 IgA response was observed in five infected fetuses, but was not detected in either 152 uninfected fetuses or in five fetuses considered as infected. The absence or presence of anti-P30 IgA antibodies in the fetus is discussed in relation to the date of maternal infection and collection of the fetal blood. It clearly appears from our study that the combined testing of both IgM and IgA in the fetus and the newborn is essential for a more efficient diagnosis of infection.

Keywords prenatal diagnosis anti-P30 IgA antibodies immunocapture ELISA acute toxoplasmosis congenital toxoplasmosis

INTRODUCTION

Toxoplasmosis, a ubiquitous protozoan infection, is caused by an intracellular parasite, *Toxoplasma gondii*. Generally benign for healthy people, it can be serious in the context of immunodeficiency, especially in the case of AIDS and of bone marrow or heart-transplanted patients or in children infected during their intra-uterine life.

The diagnosis of congenital infection at birth is classically based on the parasite detection in cord blood, amniotic fluid or placenta by cell culture or i.p. inoculation of mice [1–3] and on serological tests, which detect anti-toxoplasma IgG and IgM antibodies. In previous studies [4,5], we demonstrated that the detection of IgA antibodies directed against P30, the major *T. gondii* surface protein, could provide an additional criterion for an unambiguous diagnosis of congenital infection.

The analysis of a wider range of sera collected from newborns and pregnant women allowed us to confirm the value and reliability of the detection of anti-P30 IgA in all these cases.

Our interest was mainly focused on the detection of these anti-P30 IgA antibodies in the cord blood collected *in utero* from 162 fetuses whose mothers were in the course of acute toxoplasmosis. In addition, it was intended to evaluate this method of detection for an accurate and rapid diagnosis of an active toxoplasma infection, which would be beneficial for fetal management and prognosis.

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PATIENTS AND METHODS

Serological assays for the detection of anti-P30 IgA

The immunocapture assay used for the detection of anti-P30 IgA antibodies, derived from an immunocapture assay used for the detection of anti-P30 IgM antibodies [6,7], was carried out as previously described [4].

In our laboratory, all sera were first characterized by agglutination test with and without β mercapto-ethanol (Bio-Mérieux, Lyon, France), by a direct IgG ELISA (Diagnostics Pasteur, Marnes-la-coquette, France) and by a double-sandwich ELISA for detection of IgM antibodies to the *T. gondii* major surface protein, P30 (Platelia-Toxo IgM, Diagnostics Pasteur).

Sera from the fetuses and newborns were tested at the same time as their mothers' sera.

Human samples

This study is based on analysis of 1378 sera collected from 551 patients:

162 fetuses whose mothers had been infected around the date of conception or during pregnancy;
136 newborns whose mothers were infected during pregnancy (46 congenitally infected and 90 uninfected children);
253 women suspected of having been infected during pregnancy, including some mothers of fetuses and newborns of the previously described groups.

Fetus samples. Fetus cord blood samples were collected *in utero* between the 19th and 28th week of pregnancy.

Fetal sera were checked to be free of maternal blood, i.e. adult erythrocytes and haemoglobin A (negative Kleihauer test) and β gonadotrophin chorionic hormone. Sera were tested in parallel in maternity hospital laboratories where the parasitological assessment was performed using cell cultures and/or inoculation into mice. The detection of indirect signs of fetal infection (leucocyte, eosinophil and platelet counts, total IgM level, gamma glutamyl transferase and lactiodehydrogenase activities) was also carried out in laboratories usually associated with the maternity hospitals.

Newborn samples. Among the sera collected from the 136 newborns whose mothers had contracted toxoplasmosis during pregnancy or around the date of conception, we retrospectively tested 22 serum samples from 22 newborns (Hôpital Pitié-Salpétrière, Paris) and prospectively 591 samples from 114 children (Hôpital St Antoine and Maternité Ste Famille, Lille; CHR, Roubaix; CHR, Avranches; Maternité St Philibert, Lomme). All the newborns are over 6 months old, which was long enough to discriminate between infants congenitally infected or not, on the basis of clinical and serological follow up. In particular, a rise of specific IgG antibodies was observed after the sixth month of life in all infected children, after the disappearance of the passively transmitted maternal antibodies, whereas uninfected children were seronegative at this time.

RESULTS

Acute acquired toxoplasmosis

In 252 out of the 253 cases analysed, anti-P30 IgA were present simultaneously with anti-P30 IgM antibodies and disappeared before the latter (3–9 months). In one case, anti-P30 IgM antibodies were present but anti-P30 IgA antibodies were never detected.

Table 1 reports the results obtained with 56 sequential sera collected from nine pregnant women in the course of acute toxoplasmosis (seroconverted during gestation), without clinical signs of infection. In seven cases, anti-P30 IgA antibodies were not detected in the sera collected during the ninth month after seroconversion, whereas both specific IgG and IgM antibodies were detected.

For one patient (patient 5), anti-P30 IgM and IgA responses were weakly positive. Furthermore, anti-P30 IgA antibodies appeared 1 month after anti-P30 IgM antibodies were detectable. This patient was treated immediately anti-P30 IgM antibodies appeared, 1 month after a negative serodiagnosis.

In one case (patient 6), we noticed after 36 months a persisting high level of anti-P30 IgM and a low level of IgA antibodies. Due to the lack of serological follow up during pregnancy, the *T. gondii* infection was only detected at delivery, while a congenital toxoplasmosis was diagnosed in her newborn. Therefore, this patient never received specific treatment.

Congenital toxoplasmosis

Newborns. The detection of anti-P30 IgM and IgA antibodies has been compared in 46 congenitally infected and 90 uninfected newborns (Table 2).

The sera from two uninfected children were positive at birth (cord blood) for anti-P30 IgA antibodies (one weakly, one highly) but negative at day 7 (Table 2, footnote*). These false positive cases recorded on cord blood can be easily confirmed provided that a second sample is collected from the newborn some days after birth.

IgA^+ and IgM^- infected children (10 cases) and one child IgA^- and IgM^+ were born of mothers infected during the first or the second term of pregnancy. For the second IgA^- IgM^+ child (Table 2, footnote†), anti-P30 IgA was detected when he was 15 days old and, like IgM, IgA antibodies continued to rise: this child and his mother were infected in the last month of pregnancy.

In one congenitally infected child, anti-P30 IgA antibodies still persisted at a very high level at the age of 11 months whereas an anti-P30 IgM response was never detected. His mother was infected in the second term of pregnancy and treated with spiramycin only until delivery. Due to the absence of specific IgM at birth (anti-P30 IgA detection was not assayed at this time), this child was only treated with spiramycin. When he was 9 months old, a chorioretinitis was diagnosed, justifying a more intensive treatment (pyrimethamin and sulphadiazin).

All the 28 congenitally infected children positive for anti-P30 IgA (IgA^+) and IgM (IgM^+) antibodies at birth were born from mothers infected during the third term of pregnancy, whereas five among the six congenitally infected children negative at birth for both isotypes (IgA^- and IgM^-) were born from mothers infected during the first term. In one of these congenitally infected newborns, where anti-P30 IgM and IgA were not detected at birth, we noticed the appearance of anti-P30 IgA, but not of anti-P30 IgM antibodies, when the child was 7 months old. We also observed the rise of anti-toxoplasma IgG at this time (Table 2, footnote‡). The sixth congenitally infected child was born from a mother infected at the end of the last month of pregnancy. He was seronegative at birth (Table 2, footnote‡) but both anti-P30 IgA and IgM antibodies were

Table 1. Disappearance of anti-P30 IgM and IgA antibodies in 56 sequential serum samples from nine women infected during pregnancy

Patient no.	Term of infection	Date of sampling	IgG (U/ml)	Anti-P30 IgA	Anti-P30 IgM
1	3rd	13-8-87	25	+	+
1		24-12-87	400	+	+
1		12-4-88	1600	−	+
2	2nd	8-9-89	0	−	−
2		20-10-89	0	−	−
2		24-11-89	0	+	+
2		15-12-89	50	+	+
2		2-4-90	100	+	+
2		13-7-90	50	−	+
3	3rd	9-6-89	0	−	−
3		30-6-89	25	+	+
3		28-7-89	50	+	+
3		18-8-89	100	+	+
3		13-2-90	100	+	+
3		14-5-90	50	−	+
3		19-11-90	50	−	+
4*	3rd	6-6-90	25	−	+
4		27-9-90	200	+	+
4		23-12-90	200	−	+
5	3rd	18-1-90	0	−	−
5		19-12-90	0	+	+
5		16-3-90	0	+	+
5		6-4-90	0	+	+
5		4-5-90	25	+	+
5		5-7-90	50	+	+
5		18-10-90	50	−	+
5		31-10-90	50	−	+
5		14-12-90	50	−	+
6*	1st	4-9-86	0	−	−
6		13-4-87	800	+	+
6		15-5-87	800	+	+
6		23-9-87	400	+	+
6		13-11-87	400	+	+
6		19-12-87	400	+	+
6		22-1-88	400	+	+
6		25-3-88	200	+	+
6		24-6-88	200	+	+
6		2-12-88	200	+	+
6		23-3-89	200	+	+
6		13-12-89	200	+	+
6		19-6-90	100	+	+
6		15-1-91	100	+	+
7	1st	29-4-89	0	−	−
7		19-1-90	0	+	+
7		6-2-90	25	+	+
7		2-3-90	100	+	+
7		24-4-90	100	+	+
7		18-6-90	200	+	+
7		24-9-90	200	+	+
7		16-1-91	200	−	+
7		12-4-91	200	−	+
8	2nd	18-6-89	0	−	−
8		29-11-89	100	+	+
8		15-2-90	100	+	+
8		12-5-90	100	+	+
8		11-10-90	50	−	+
9	2nd	5-8-88	0	−	−
9		29-12-88	400	+	+
9		18-1-89	400	+	+
9		6-4-89	200	−	+

* Congenitally infected children were born from these two mothers.

Table 2. Detection of specific anti-P30 IgM and IgA antibodies at birth in 46 infected (CT^+) and 90 uninfected (CT^-) children

	Anti-P30 IgA^+		Anti-P30 IgA^-	
$n=136$	CT^+	CT^-	CT^+	CT^-
Anti-P30 IgM^+	28	2*	2†	2
Anti-P30 IgM^-	10	0	6‡	86

* One child was highly and one weakly positive at birth in the cord blood but negative at day 7.

† One child was weakly positive for IgM at birth and highly positive for IgM and IgA at day 15.

‡ One child was positive for IgM and IgA at day 20. One child was positive for IgA but not for IgM at 6 months.

present in the serum collected at day 20 and continued to rise in parallel; anti-P30 IgM antibodies were absent from the sample collected in the child when he was 6 months old whereas anti-P30 IgA antibodies were still detected at this time.

In the other cases, anti-P30 IgM and IgA antibodies, when detected in congenitally infected newborns, disappeared in 1–5 months, depending on the antibody level at birth.

Fetuses. Among the sera collected from 162 fetuses, 16 were positive for anti-P30 IgA antibodies, but a contamination with maternal blood was noted in nine sera from five fetuses. The seven true IgA† sera proved to be from five fetuses considered as infected (Table 3).

One-hundred and fifty-seven fetuses were negative for IgA antibodies, 152 of these were negative for toxoplasma infection on both serological and clinical follow up. Congenital toxoplasma infection was suspected or proven in the remaining five cases by clinical, parasitological or serological signs (Table 4).

We analysed at birth the sera from 37 newborns tested during their intra-uterine life and then followed the subsequent serological and clinical evolution. Except for two cases, all were considered as uninfected with *T. gondii.* The two congenitally infected children were born from mothers infected in the first term of pregnancy and one was negative both *in utero* and at birth for anti-P30 IgM and IgA antibodies (Table 4, case 5). Interestingly, the other child was IgM^- but IgA^+ at week 22 of his intra-uterine life and at birth, whereas the other signs of fetal infection were not present (Table 3, case 5).

DISCUSSION

This study first extends our previous work [4,5] and confirms the value of measuring the IgA antibody response directed against P30, the major surface antigen of *T. gondii*, in congenital and acute acquired toxoplasmosis. Similar results were recently described by Huskinson *et al.* [8]. In addition, the demonstration that an anti-P30 IgA antibody response can be observed in the fetuses infected by *T. gondii* suggests that this immunocapture test has additional usefulness in the prenatal diagnosis of congenital toxoplasmosis.

During the acute phase of toxoplasmosis, we detected IgA antibodies directed against P30 in sera of all patients, sometimes later than specific IgM but always earlier than specific IgG. In the majority of cases, when IgG continued to rise and IgM antibodies persisted ('residual' IgM), IgA antibodies disap-

Table 3. Fetuses with anti-P30 IgA antibodies proven to be infected by toxoplasma

Case	Term of infection	Date of punction (week)	Clinical signs	Specific IgM	Indirect signs	Culture or inoculation	Evolution
1	1st	24	–	–	GGT ↗, LDH ↗ Eos. (13%)	–	
		27	–	–		–	
		31	Hydrocephaly	+		–	Pregnancy termination
2	2nd	25	Ascitis, ventricular dilatation, hepatosplenomegaly	+	–	–	Pregnancy termination
3	1st	23	–	+	–	–	
		25	–	+	–	+	Pregnancy termination
4	1st	24	–	+	–	–	Pregnancy
		25	–	+	–	+	termination
5	1st	22	–	–	–	–	CT+*

* Congenital toxoplasmosis-infected. Antibody rise at 6 months after birth.
GGT, gammaglutamyltranspeptidase; LDH, lacticodeshydrogenase; Eos., eosinophilic polymorphonuclear cells.

Table 4. Fetuses without anti-P30 IgA antibodies either suspected or proven to be infected by toxoplasma

Case	Term of infection	Date of punction (week)	Clinical signs	Specific IgM and indirect signs	Culture or inoculation	Evolution
1	1st	28	Growth retardation	–	–	Pregnancy termination
2	1st	24	Growth retardation	–	–	Pregnancy termination
3	1st	29	–	–	+	Pregnancy termination
4	2nd	22	–	–	+	Pregnancy termination
5	1st	20	–	–	–	CT+*

* Congenital toxoplasmosis-infected. Antibody rise at 6 months after birth.

peared earlier (between 3 and 9 months) and were not detected in the chronic phase of toxoplasmosis, as described in other studies focused on the anti-toxoplasma IgA response [4,8–17]. We observed only a few exceptions, corresponding to untreated patients, suggesting that the treatment, decreasing the antigenic stimulation, could influence the antibody kinetics. Thus, the parallel detection of the three isotypes, IgG, IgM and IgA, is of major value in determining the phase of infection, especially during pregnancy. If specific IgM alone is detected, the analysis of a second sample after 3 further weeks of evolution will confirm (presence of IgA) or not (absence of IgA) the possibility of a recent infection and, when specific IgM and IgA are simultaneously detected, the patient must be considered to be in the course of acute toxoplasmosis. As shown by the number of studies focused on the subject [10,18–22], the early diagnosis of acute toxoplasmosis in pregnancy is of utmost importance. Indeed, pregnancy and fetal management are very different depending on whether the acute phase occurred after the date of conception, when the risk of fetal infection exists, or before the date of conception [5,23,24]. In the latter case, there is no risk of congenital infection, except in exceptional cases, when the woman is immunocompromised [25].

In congenital toxoplasmosis, anti-P30 IgA antibodies are found more frequently than anti-P30 IgM in infected fetuses and newborns. The presence of specific IgA definitively proves the congenital infection since, like IgM, IgA does not cross the placental barrier. In relation to the progressive development of the fetal immune system, anti-P30 IgM and IgA detection is more significant in the case of a late infection, except if infection is contracted in the last month of pregnancy: in this case,

additional serodiagnosis of toxoplasmosis must be performed after 1 month of life as the newborn may be seronegative at birth, especially if the maternal serodiagnosis was positive [26]. As observed in one case of a congenitally infected child, the late appearance of anti-P30 IgA antibodies (absent at birth, as well as anti-P30 IgM antibodies) can constitute an additive criterion of infection.

Interestingly, anti-P30 IgA can be detected in fetuses as early as the second term of pregnancy provided that maternal infection was acquired early after conception. This observation is fascinating from the immunological point of view. Indeed, in the absence of antigenic stimulation, the production of all isotypes of immunoglobulins is non-existent in the human fetus; in particular the level of IgA in 1-year-old children is only 20% of that observed for adults [27,28]. Although the specific IgA response is generally weaker in fetuses than in newborns, our data demonstrate that toxoplasma infection can evoke production of specific IgA by the fetal immune system.

The anti-P30 IgA response can disappear before birth, showing the importance of the prenatal diagnosis. Fetal blood samples must be collected in the second term of pregnancy (when the fetal immune system is functional) by experienced obstetrical teams [29]. Several biological tests must be performed to verify that fetus samples are not contaminated with maternal blood, which would interfere with diagnosis of congenital infection on the basis of parasitological and serological results [1,2,30–32].

In the case of negative results, fetal infection cannot be excluded; in particular it could be possible that the cord blood was collected before the fetus infection. The classical survey and treatment of the pregnant women by spiramycin must be continued until delivery.

In the case of positive results, clinical and ultrasound survey must be intensified and the termination of pregnancy has to be discussed if clinical signs of congenital infection are obtained (hydro- or microcephaly). When the continuation of pregnancy has been decided, the administration of spiramycin must be replaced in the third term by a sulphamide and antifolic combined treatment in order to effect greater inhibition of toxoplasma proliferation in the fetal tissues [33–35].

This work confirms the diagnostic interest of anti-P30 IgA in acute acquired and congenital toxoplasmosis. In acquired toxoplasmosis, the majority of the patients are positive for anti-P30 IgA antibodies during the acute phase of infection, simultaneously with specific IgM in the majority of cases, whereas they are negative in the chronic phase, when specific IgM could still be detected ('residual' IgM). Thus, the testing of both IgM and IgA antibodies has proven reliable and of great help to diagnose a recently acquired toxoplasmosis, which is important for a better management of pregnancies. In congenital toxoplasmosis, anti-P30 IgA was found in infected newborns more frequently than anti-P30 IgM.

Moreover, we demonstrate here the possibility of detection of anti-P30 IgA in the infected fetus as early as week 23 of pregnancy, which is of considerable interest for a better understanding of the fetus immune system function. Our data point out the value of the detection of anti-P30 IgA in the antenatal diagnosis of congenital toxoplasmosis. It appears from our study that the combined testing of both IgM and IgA in the fetus and the newborn is essential for a more efficient diagnosis of congenital infection.

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